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Submitting Official:	Francis J. Rattay
Title:	Manager, Regulatory Affairs
Address:	Mobay Road Pittsburgh, Pa 15205
Telephone No.:	(412) 777-7471
FAX No.:	(412) 777-7484

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If you have any questions, please contact me.

Sincerely,

Francis J. Rattay

Francis J. Rattay
Manager, Regulatory Affairs
(412) 777-7471

Attachment
Certified Mail No.: P 827 2. 581

THE TOXICOLOGY OF MDI

A Paper Intended for Submission

to Critical Reviews in Toxicolcgy

First Draft Prepared

by

M H Litchfield

Melrose Consultancy

Arundel, UK.

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THE TOXICOLOGY OF MDI

ABSTRACT

Monomeric and polymeric MDI are used for the production of polyurethane materials. They have been shown to be slight to moderate skin and eye irritants and strong skin sensitisers in animal models. A range of inhalation studies has been carried out to assess the effect of acute and repeated MDI exposures. The principal toxic effect in laboratory animals is an irritant action of the respiratory tract leading to severe respiratory distress at acute exposure levels. A 4 hr.-LC_{50} of 490 mg/m^3 was established for polymeric MDI aerosol in the rat. With repeated exposure to lower levels polymeric MDI increases lung weight and causes histopathological changes in the lung, nasal cavity and mediastinal lymph nodes in rats. In a 2 year chronic rat study these changes were seen as yellowish macrophage accumulation in the lung and mediastinal lymph nodes and basal cell hyperplasia in the nasal cavity at exposure levels of 1 and 6 mg/m^3. A small number of pulmonary tumours were also observed at the top exposure level of 6 mg/m^3. The non-neoplastic changes were seen as a direct result of the irritant action of MDI, leading to the production of the pulmonary tumours by a non-genotoxic process. An overall no effect level of 0.2 mg/m^3 was established for this study. Genetic toxicity assays to various end points in vitro gave conflicting results but an in vivo mouse micronucleus assay showed MDI to be non-mutagenic.

A. INTRODUCTION

Methylene diphenyl diisocyanate (MDI), in its monomeric and polymeric forms, is used to produce polyurethane foams, elastomers, coatings, adhesives and elastomeric fibres by reaction with polyhydroxy compounds. About 95% of the production of polyurethanes is based upon the use of MDI or toluene diisocyanate (TDI). The world production figures are about a million tonnes annually for both MDI and TDI[1]. Although much of the production and use of MDI is under carefully controlled automated factory conditions, its use to prepare polyurethane foams in situ by spray techniques can lead to greater human exposure potential.

Because of its large scale use and potential for human occupational exposure the toxicology of MDI has been investigated by a series of studies commencing in the 1960s and continuing to the present time. This review encompasses all the relevant available toxicological information on the compound and addresses the current evaluation of its potential hazard.

B. PHYSICO-CHEMICAL CONSIDERATIONS

An understanding of the various physical forms of MDI and pertinent chemical reactions is important in order to undertake toxicological tests appropriately and to evaluate the results of studies. MDI is available in several forms based upon purified monomeric MDI and polymeric MDI.

Pure MDI is composed mainly of the 4,4'-isomer (>95%) and usually contains a small amount of the 2,4'-isomer. It is a white-pale yellow solid of melting point 38°C. and with a vapour pressure of 7×10^{-6} mmHg at 20°C. It is virtually insoluble in water (0.2 g./100 g. at 20°C. but is highly soluble in many organic solvents. MDI reacts slowly in water below 50°C. to form polyureas. It has been shown that MDI and other isocyanates react rapidly with moisture present in solvents such as DMF and DMSO[2,3]. This information is pertinent when assessing the results of toxicological studies where MDI has been dissolved in these solvents and this will be commented upon again at the appropriate points in this review. The difficulties with handling pure MDI in commercial use and its tendency to form dimers at temperatures >40°C. have led to the development of modified MDIs which are liquid at ordinary temperatures and are not prone to dimerisation. More than 80% of MDI usage is in its polymeric form. Polymeric MDI contains a large proportion of monomeric MDI, usually in the range 40-60%, together with other MDI isomers, dimers and polymers. It is a dark amber coloured, viscous liquid of boiling point 330°C. at 780 mmHg. Its vapour pressure is determined largely by its monomeric MDI content.

Because of its low vapour pressure, very little MDI will be present in the atmosphere as vapour at ambient temperatures (<0.05 mg/m^3). In commercial uses such as foam blowing, MDI may be present also in aerosol form in the atmosphere. These points should be kept in mind when evaluating the results of inhalation toxicity studies. The reports from some of the older studies state that MDI was generated as a 'vapour', but the concentrations used indicate that this could not have been the case. It is likely that the atmospheric MDI in these tests was in a condensed vapour aerosolised form. Such cases will be indicated where appropriate in this review. It should be noted that in the more recent inhalation studies, polymeric MDI has been used as the test material, reflecting its greater commercial usage, and that attention has been paid to the generation of atmospheres in aerosol form with adequate monitoring of MDI concentrations and aerosol particle size.

C. MODE OF ACTION AND PHARMOCOKINETICS

The most important site of action of MDI and other isocyanates is the respiratory tract. They have a direct irritant effect on the respiratory mucosa, the response to which is due to the triggering of normal protective mechanisms of the upper respiratory tract. This response is similar to that of other respiratory irritants and, in humans, results in increased secretion, cough, pain on respiration and, if severe enough, some restriction of air movement due to secretion, edema and pain. The signs of toxicity in laboratory animals are documented in the results of inhalation studies described in the following sections of this review.

Another type of response, which can develop in a small proportion of exposed humans, is pulmonary hypersensitivity which is shown either as isolated bronchospactic reaction or as an asthmatic-type response to low non-irritating concentrations of the inhaled isocyanate. Symptoms include immediate or late onset of reduced pulmonary function and development of airway hyperreactivity. Immunoglobulin E (IgE) class antibodies have been detected in the serum of individuals with isocyanate lung sensitivity. Many studies have been undertaken to identify the causative mechanism(s) of isocyanate hypersensitivity, including clinical and epidemiological surveys and the use of animal models such as the guinea pig. At present the mechanism is still unclear but there is evidence for an immunological aetiology[4].

A study has been undertaken to assess the uptake, distribution and fate of monomeric MDI after inhalation exposure of the radiolabelled compound[5]. Male 300 g. rats were exposed to ^{14}C-MDI in aerosol form (particles <5 µm.) for 15 min. The MDI atmosphere level was not measured but the dose to each rat (0.0127-0.0260 ng.) was calculated from the total radioactivity recovered from each animal in excretory products and tissues. Radioactivity in whole blood and plasma was highest in the first 24 hr. and then showed a slow decline over the remaining sampling period up to 96 hr. Excretion via the urine and faeces was fairly rapid with 70% of the activity eliminated in 96 hr., together with about 2% expired as $^{14}CO_2$. The greatest concentration of radioactivity found in tissues over the 96 hr. sampling period was in the lungs. Since a significant proportion of the total dose was found in the gastrointestinal tract, then some of the MDI might have been ingested. Because about 20-25% of the radioactivity remained in the animals 4 days after the exposure and since blood levels showed a slow decline during this time, then the pharmocokinetics of MDI may be somewhat complex and require multicompartmental analysis for further assessment.

A further insight into the pharmacodynamics of MDI may be gained from studies with the related diisocyanate TDI. When the radiolabelled compound was exposed to rats at 2 ppm as vapour for 4 hr., the recovery of radioactivity after 48 hr. was 14.9% in urine, 47.3% in faeces and 34.1% in the carcass. In tissues, the greatest concentration of activity was found in the lung[6]. Following 4 hr. exposures at ^{14}C-TDI vapour concentrations ranging from 0.00005-0.146 ppm the uptake of radioactivity into blood of guinea pigs was linear during expocure and continued to increase slightly post-exposure. A comparative study with methyl isocyanate indicated that the uptake into arterial blood is a function of exposure concentration and is independent of isocyanate structure[7]. Further studies with rats showed that these relationships also held for this species[8]. The majority of the ^{14}C-label in blood plasma was shown to be associated with high molecular weight components (>10 kDa) and the predominant conjugate had a relative molecular weight of 70 kDa.

D. ACUTE TOXICITY

The various forms of MDI appear to have low acute oral and dermal toxicity. Studies on polymeric MDI at single oral doses up to 10,000 mg./kg. or with a modified liquid MDI at 5,000 mg./kg. gave no mortalities and little or no other effect in rats[9,10]. Similarly the same substances as a single application to intact or abraded rabbit skin at doses up to 9.4 g./kg. (polymeric MDI) or 2 g./kg. (modified liquid MDI) did not produce any effects during a subsequent 14 day observation period apart from slight skin irritation[11,12]. Similar results at high doses have been reported by Woolrich[13] and support the view that monomeric and polymeric MDI have very low acute oral and dermal toxicity.

The acute inhalation toxicity of monomeric MDI has been studied in groups of 6 male rats for a 1 hr. exposure period followed by 14 days observation[14]. The concentrations of MDI, which were said to be in 'vapour' form, ranged from 0.6-1,530 mg/m^3. Slight restlessness and erythema were observed at 0.6 mg/m^3, with additional signs at the next level of 80.8 mg/m^3 of salivation, lacrimation, nasal drip and escape behaviour. At 186 mg/m^3, 4 out of 6 rats died within 26 hr. and all rats died at exposure levels of 562 mg/m^3 and above. Lung congestion and oedema was present upon gross examination of rats exposed to 186 mg/m^3 and above. The approximate 1 hr.-LC_{50} was estimated to be 178 mg/m^3

The acute inhalation toxicity of polymeric MDI was assessed using groups of 10 male and 10 female rats exposed to the test material as an aerosol (>95% particles <5 μm.) for 4h at concentrations of 384-523 mg/m^3 followed by a 14 day observation period[15]. The atmospheres were sampled by cascade impactor or glass filters and analysed by spectrophotometry or HPLC. Laboured respiration was the main clinical sign in all groups and deaths occurred within 2 days of exposure. Animal bodyweight decreased over the first 2-4 days after exposure and then increased again in survivors. Gross examination of rats dying due to exposure showed lung haemorrhage in animals at the higher concentrations. A 4 hr.-LC_{50} of 490 mg/m^3 was established.

In another study on polymeric MDI, said to be in 'vapour' form, rats (sex unspecified) were exposed to concentrations ranging from 1-280 mg/m^3 for 6 hr. followed by a 15 day observation period[16]. The test atmospheres were sampled by impinger followed by colorimetric analysis. 4 out of 6 rats died at 23 mg/m^3 and all rats died at the next concentration of 38.5 mg/m^3 and above within 24 hr. Rats at 23 mg/m^3 showed laboured breathing which was of increased severity at the higher concentrations. Gross examination of rats at 1 and 2 mg/m^3 revealed slight lung haemorrhage while at the higher concentrations there was increased exposure related lung haemorrhage and congestion.

The pulmonary and sensory irritant potential of monomeric MDI was studied in male mice by inhalation exposure[17]. The mice, 4 per group, were exposed, head only, to the compound in aerosol form (mmad 0.7 μm.) for 4 hr. at concentrations ranging from 6.7-58.5 mg/m^3. The atmosphere concentrations were analysed by a gravimetric method after sampling by Teflon membrane filters. The respiratory rate of the mice, measured before and during exposure, showed a concentration and time response relationship. During exposure there was a small initial increase in respiration rate followed by a decrease, a pattern typical of a pulmonary irritant. The decreased rate was minimal at 6.7 mg/m^3 but was rapid and marked at 58.5 mg/m^3. Lung weight increased, also in a concentration dependant manner, with a 10% increase at the lowest level and a 42% increase at the highest. The MDI aerosol concentration required to reduce the respiratory rate by 50% was 32 mg/m^3. There was little indication that MDI possessed sensory irritation potential.

The skin and eye irritant potential of monomeric and polymeric MDI has been assessed in several studies using the rabbit. Dermal application (0.5 ml.) to intact or abraded skin followed by 24 hr. under an occluded dressing and then assessments 24, 48 and 72 hr. later showed monomeric MDI to be a slight irritant and a modified liquid MDI to be a moderate irritant using the Draize scoring system[18,19]. In the eye tests, 0.1 ml. of the test substance was placed in one eye of each rabbit, the other eye acting at the control, and assessments made for several days thereafter. A technical monomeric MDI showed moderate conjunctival hyperemia after 24 hr. and slight epithelial alteration after 7 days. All changes had resolved within 10 days and the compound was considered to be a slight eye irritant on the Kay & Calandra scale[18]. A modified liquid MDI did not cause any corneal injury and produced only mild iridal and conjunctival irritation at 1h post-instillation[20]. The iridal irritation had subsided by 24 hr. but the conjunctival irritation persisted for 16 days. All eyes were normal at 21 days post-installation. The test substance was classified as mildly irritating to rabbit's eyes on grading and scoring with the Draize procedure. Polymeric MDI produced corneal effects within 24 hr. of instillation which quickly repaired, the severity of irritation was judged to be moderate on the Draize scoring system[21].

The skin sensitisation properties of monomeric MDI have been assessed in several studies using animal models. It was shown to be a strong skin sensitiser in a Magnusson and Kligman guinea pig test[18]. A dose of 0.05 ml. 5% MDI in olive oil or Freund's adjuvant was given i.d. followed by a week's rest. Then a dorsal application of 25% MDI in Vaseline was made followed by another week's rest. In the third step MDI at concentrations of 1-20% in Vaseline was applied to the animals' flanks for a 24 hr. period and the skin assessed 24 and 48 hr. later. Triggering action was then evaluated by further application of 10% MDI in Vaseline at 3, 5, 7 and 10 weeks, or by 10% TDI at 12 weeks. The strong sensitising action of MDI was maintained over the periods of the triggering applications and cross sensitivity was shown with TDI.

In two other studies assessing skin sensitisation the mouse ear was used as the model[22,23]. Mice were treated with MDI solutions (in ethyl acetate or acetone) either on the shaved back or abdomen and then challenged with MDI solutions on the ear 4 or 7 days later. Ear thickness was then measured at time intervals up to 24 or 72 hr. after the challenge. Marked ear swelling occurred in both studies. In one of the studies[23] the MDI response showed a dose related effect up to 37 mg/kg. and the dose which produced sensitisation in 50% of the mice was 0.73 mg/kg. Cross-sensitisation with TDI was shown in both studies.

The influence of the solvent used when testing MDI skin sensitisation was evaluated in another study[24]. MDI was dissolved in a range of organic solvents and then applied to the shaved backs of mice which were then challenged later with a dose applied to the ear. The strongest responses, as measured by ear thickness, were from MDI dissolved in ethyl acetate, acetone, toluene or dichloromethane. Little or no response was obtained with MDI dissolved in DMF or DMSO and this could be a reflection of the interaction of MDI with moisture in these solvents mentioned earlier (Section B).

E. **REPEATED DOSE TOXICITY**

Male and female rats, 10/sex/group, were exposed by inhalation to aerosol (>95% particles <5 µm.) concentrations of polymeric MDI at 0, 2, 5 or 15 mg/m^3 for 6 hr./day, 5 days/week, for 2 weeks[25]. MDI atmosphere concentrations and particle size were monitored by quartz crystal microbalance (QCM) cascade impactor throughout the study. Rats were observed before and after each day's exposure and once per day during the 2 day non-exposure interval. Bodyweight was measured before exposure, on days 3, 6, 8 and 10 of exposure, and before sacrifice. Gross examination was undertaken on all rats dying or killed at termination and lungs were weighed. 7 males and 1 female died at the top exposure level of 15 mg/m^3, the first death occurring at 4 days. There were no mortalities at the lower levels. There were no abnormal clinical signs for the first 3 days at 15 mg/m^3 but then there were increasing signs of piloerection and respiratory tract irritation, which were more pronounced in males than females. There were similar but milder effects at 5

mg/m^3 and none at 2 mg/m^3. Bodyweight gain was severely depressed at the top exposure level in both sexes but a statistically significant decrease occurred only in males at 5 mg/m^3. A dose related increase in lung/bodyweight ratio occurred at all exposure levels in both sexes. No abnormalities due to exposure were seen upon gross examination. Since a small increase in lung/bodyweight ratio was the only effect seen at 2 mg/m^3 then the no observed effect level (NOEL) was close to but lower than this concentration.

In a second study with polymeric MDI aerosol rats were exposed to a single concentration of 15 mg/m^3 for 6 hr./day, 5 days/week, for 2 weeks[26]. Two groups of 20 males, 20 females were used, one group with rats starting at 4 weeks old, the other with rats at 6 weeks old. The animals were observed only for mortality. The group of rats starting at 4 weeks old were the more susceptible to MDI exposure with 15 males and 20 females dying during the study, with the first death at Day 4 in males. 12 males and 1 female died in the group starting at 6 weeks old, with the first death at Day 8 in males.

A study was set up to assess the effect of polymeric MDI 'vapour' on rats exposed for 30 min./day, 5 days/week, for 2 weeks[27]. It was not possible to completely vapourise the test substance and consequently exposure concentrations were much less than those intended. The measured exposure levels were 2, 6.7 or 26.8 mg/m^3. No effects were seen on several parameters observed or measured, including clinical signs, bodyweight, haematology and lung, liver and kidney histopathology.

A 90 day study with monomeric MDI has been briefly described in an abstract[28]. The MDI was generated in aerosol form and rats were exposed to concentrations of 0.3, 1.0 and 3.0 mg/m^3 for 18 hr./day, 5 days/week. The atmospheres were monitored by a variety of methods. Effects were reported at

1.0 and 3.0 mg/m^3 as slightly lower bodyweight and an increase in wet and dry lung weight. Submucosal infiltration of mononuclear cells, goblet-cell hyperplasia and erosion of respiratory epithelium in nasal and paranasal sinai and hyperplasia of the bronchus associated lymphatic tissue and inflammatory alteration in the lungs was seen at these two levels.

Two subchronic studies were undertaken with polymeric MDI in aerosol form (95% particles <5 μm.). The first study [29] was initiated at nominal exposure concentrations of 0, 0.2, 1 or 5 mg/m^3. During the study atmospheric MDI measurements using a variety of methods gave conflicting results and a subsequent investigation indicated that the actual concentrations may have been somewhat higher (0.35, 1.4 or 7.2 mg/m^3). Groups of 15 male, 15 female rats, 6 weeks old at start, were exposed to the atmospheres for 6 hr./day, 5 days/week, for 13 weeks. Comprehensive assessments included haematology, blood and urine biochemistry and microscopic examination of tissues at termination. Apart from a slight effect on male bodyweight at the top dose there were no other biologically significant changes due to exposure. This was contrary to the results of the subacute study described previously[25] where definite effects were evident at 5 mg/m^3.

The second subchronic study[30] with polymeric MDI was set up with exposure levels of 0, 4, 8 or 12 mg/m3. MDI atmosphere concentrations were measured by gravimetric analysis after collection on glass filters, 5 times each exposure day, and the results were close to the nominal values. Groups of 30 male and 30 female rats, 6 weeks old at the start, were exposed to the test atmospheres for 6 hr./day, 5 days/week, for 13 weeks. They were assessed for a comprehensive range of parameters as described in the previous study. At the end of the 13 week exposure period, 20 males and 20 females at 12 mg/m3 and 15 males and 15 females at each of the other levels, were terminated for gross and microscopic examination of tissues. The remaining animals were held for a further 4 week unexposed recovery period.

11 males and 4 females died at 12 mg/m^3, the first death occurring during Week 3. Signs of severe respiratory distress were seen at this level during exposure, with gradual recovery post-exposure. There were less severe signs of respiratory effects at 8 mg/m^3 and none were seen at 4 mg/m^3. A depression in bodyweight gain in the rats at the top level during exposure was reversed during the 4 week recovery period. Smaller, dose-related bodyweight gain depressions occurred at the two lower levels. Lung/ bodyweight ratio was increased in rats exposed to 8 or 12 mg/m^3 compared to controls. Treatment related histopathological changes occurred in the nasal cavity, lungs and mediastinal lymph nodes in males and females surviving to termination in a dose dependent manner at all exposure levels. These changes included olfactory epithelial atrophy of the nasal cavity and accumulation of alveolar macrophages in the lungs. No such changes were seen in rats that died or were killed in extremis. The effects were slight at 4 mg/m^3, which was probably close to a NOEL.

Despite some differences in the effect levels in the two subchronic studies described above, the results provided sufficient information for setting the exposure levels for a chronic toxicity/carcinogenicity study on polymeric MDI. The long term study [31] was undertaken with 60 male and 60 female rats, 6 weeks old at the start, at aerosol (95% particles, <5 μm.) exposure levels set at 0, 0.2, 1 or 6 mg/m^3. Exposure was for 6 hr./day, 5 days/week for 2 years. Satellite groups of 10 males and 10 females were exposed for 1 year. Rats were observed and weighed regularly throughout the study and subjected to routine haematological and biochemical assays. Organ weights were measured and tissues examined microscopically at the 1 and 2 year terminal sacrifices. The MDI atmosphere levels were monitored regularly by ß-attenuation with checks by gravimetric analysis. Measured exposure levels were close to the nominal values.

No treatment related changes were seen for clinical condition, bodyweight gain, haematology or blood and urine biochemistry. Mortalities in the exposed groups were comparable to those in the control group over the 2 year period. Lung/bodyweight ratio was increased in rats exposed to 6 mg/m^3 at 1 and 2 years. Dose related changes occurred in lungs, mediastinal lymph nodes and nasal cavity of rats at 1 and 6 mg/m^3. These effects were evident at 1 year and well established at 2 years. The main changes in the lungs were described as yellowish macrophage accumulation, alveolar duct epithelialisation and localised fibrotic change. The main changes in the mediastinal lymph nodes were described as yellowish macrophage accumulation and in the nasal cavity as basal cell hyperplasia. It would appear that these non-neoplastic changes were mediated via the irritant properties of polymeric MDI. Neoplastic findings are described in Section G. on carcinogenicity. There were no effects at the lowest exposure level of 0.2 mg/m^3 and this was established as the NOEL for the study.

F. GENETIC TOXICITY

Monomeric MDI has been tested for genotoxic potential in several in vitro assays to different end points. When assessing the results the fact that DMSO was used as the solvent for MDI in these studies has to be kept in mind. As indicated in Section B., MDI is unstable in the presence of moisture in solvents such as DMSO and DMF.

MDI was evaluated in 5 bacterial assays using up to 5 strains of Salmonella typhimurium in the presence or absence of a metabolic activating system. Positive responses were obtained in four studies[13,32,33,34] with the TA98 and TA100 strains in the presence of metabolic activation, while MDI was not mutagenic in these strains in the fifth study[35]. Positive responses were not obtained in the assays using TA1535, TA1537 or TA1538 with or without metabolic activation. MDI was not mutagenic when incubated at concentrations of 2.5-250 µg./ml. in mouse lymphoma suspensions for 3 hr. at 37°C. in the presence or absence of metabolic activation[36,37]. It increased the transformation frequency of Syrian hamster kidney BHK 21 C13 cells when incubated in the cell suspensions at 125-2,000 µg./ml. in the presence or absence of metabolic activation[38,38].

In an in vivo study, 10 week old male mice were given monomeric MDI by i.p. injection at single doses of 32, 80 or 200 mg./kg. using 6 mice per group[40]. 24 hr. later the mice were killed by cervical dislocation, the femur excised and bone marrow cells collected for staining and examination. Erythrocytes were observed for the incidence of micronuclei and no differences were seen between the MDI and negative control groups. The positive control group, dosed with triethylenemelamine (TEM), gave the expected increase in micronuclei incidence. MDI, therefore, showed no potential for the induction of chromosomal aberrations and was not mutagenic in this in vivo study.

G. CARCINOGENICITY

The carcinogenic evaluation of polymeric MDI was undertaken in the long term inhalation rat study described in Section E.[31]. Groups of 60 male and 60 female rats were exposed for up to 2 years to concentrations of 0, 0.2, 1 or 6 mg/m^3 polymeric MDI. The study was carried out without any major difficulties. The overall mortality at the end of 2 years was 30-40% for males and 17-32% for females, thus allowing sufficient numbers of rats for terminal examination. There were no significant differences in mortality between the control and test groups. Microscopic examination of tissues revealed non-neoplastic findings at 1 and 6 mg/m^3 in lungs, mediastinal lymph nodes and nasal cavity as described previously. In addition, at the highest dose only, there were six lung adenomas (6/60) and one adenocarcinoma (1/60) in males and two lung adenomas (2/59) in females. No pulmonary tumours were found in animals at the two lower exposure levels. The production of the small number of pulmonary tumours at the top dose level was considered to be derived from the irritant properties of polymeric MDI by a non-genotoxic mechanism.

H. HUMAN TOXICITY

Human responses to occupational exposure to isocyanates have been described in detail in the scientific literature. Since adequate recent reviews have appeared on this subject[41,42] only the salient features are summarised here.

There are very few reports on the effects of MDI due to short term exposure. Some indication is given by the description of symptoms in 12 men standing 20-40m. from an MDI spray application[43]. Symptoms developed from one hour to several hours after exposure to the mist and included asthmatic breathing, constriction of the chest and coughing. All had recovered within 10 days of exposure.

More information is available for longer term occupational exposure to low levels of atmospheric MDI although there are no reports of good epidemiological studies. The main effect reported in these conditions is the onset of respiratory sensitisation in a small number of individuals. At first the symptoms resemble those of a cold or mild hay fever. However, more severe asthmatic symptoms can develop with time. The effects may subside after a few hours without exposure to MDI. Sensitised individuals who continue to work with MDI may develop symptoms more quickly after exposure and the number and severity of the effects may also increase. It is probable that an exposure to a higher concentration of MDI is needed to sensitise certain individuals. The condition then develops after days, months or even years of working with the compound. Longer term exposure may also progressively decrease lung capacity more than normal.

I. DISCUSSION AND CONCLUSIONS

The toxicological evaluation of MDI has centred on its effect upon the respiratory tract where it has a direct irritant action in humans and laboratory animals, as with other isocyanates. After inhalation exposure, MDI is rapidly absorbed, distributed and excreted, followed by a second slower stage of elimination. Radiolabel studies with the related compound TDI showed that the highest relative activity was associated with the respiratory tract in rats and guinea pigs and that radioactivity in blood plasma was associated with a high molecular weight component.

The first observable signs of the acute respiratory irritant effect are seen at levels from about 1 mg/m^3 upwards in laboratory animals. The toxic effects occur at similar exposure levels for both monomeric and polymeric MDI. The consequences of the toxic irritant action can be followed through the subacute, subchronic and chronic rat studies with polymeric MDI. In a subacute 2 week study, increased lung weight occurred at levels down to 2 mg/m^3. In a 13 week study, treatment related histopathological changes occurred in the nasal cavity, lungs and mediastinal lymph nodes at exposure levels of 4 mg/m^3 and upwards. Similar changes were seen in the 2 year study with increased lung weight at 6 mg/m^3 but not at the lower level of 1 mg/m^3. Dose related histopathological changes were seen in the nasal cavity, lungs and mediastinal lymph nodes at 1 and 6 mg/m^3. A small number of pulmonary tumours were found in animals at the top exposure level of 6 mg/m^3. A detailed examination showed that these tumours developed in the presence of macrophages and epithelial erosions at the alveolar duct regions of the lung. The recurrent epithelial erosion resulted primarily in proliferation of Type II pneumocytes which progressed to tumours. Aggregates of macrophages containing the test material were frequently associated with the pneumocyte proliferation. Therefore, it would seem that the pulmonary tumours developed secondarily to the irritation by polymeric MDI aerosol. This kind of change, involving Type II alveolar cells, is a common non-specific reaction to many forms of toxic lung injury. The processes involved can produce tumours through non-genotoxic (epigenetic) mechanisms. This is supported by the findings at 1 mg/m^3 where there was minimal irritation, non-neoplastic lung changes were slight and no pulmonary tumours were found. Thus exposure to polymeric MDI at levels which do not result in recurrent tissue damage should not produce tumours. An overall NOEL of 0.2 mg/m^3 was established in the study.

The information from genotoxicity testing is not consistent for monomeric MDI. In particular in vitro assays to various endpoints produced conflicting results. DMSO was used as the solvent in these tests and it is known that unless moisture is excluded, MDI reacts rapidly in this solvent to give a variety of products. The results, therefore, may not be due to MDI itself.

In the in vivo mouse micronucleus test there was no evidence that MDI caused chromosomal aberrations. There is, therefore, no evidence that MDI is capable to mutagenic action in the intact mammalian system.

The other major effect of MDI upon the respiratory tract is a hypersensitivity response. This is shown in a small proportion of occupationally exposed humans, usually after working with the compound over a long period of time. Investigation of human subjects and supporting work in animal models indicate that an immunological mechanism is involved although the underlying pathogenesis of isocyanate hypersensitivity still remains unclear.

In setting safe occupational exposure limits for isocyanates, their propensity to cause respiratory sensitisation and to decrease lung capacity in humans has been the main criteria considered by the relevant authorities. The levels at which these effects occur over long time periods are lower than those for other effects seen in humans or in animal inhalation studies. When considering the evidence available from human exposure to MDI, the American Conference of Governmental Industrial Hygienists considered that there was not sufficiently good data to link occupational atmosphere levels with effects on respiratory tract sensitisation and pulmonary function decrement. Instead they considered that MDI was sufficiently similar in toxic action to TDI which had a better human exposure data base upon which a Time Weighted Average (TWA) limit of 5 ppb had been set. Under these circumstances they assigned the same TWA to MDI[44]. This standard is achievable on plants where proper work practices and ventilation are provided and maintained and will ensure that normally healthy workers suffer no ill-effects. Many countries have adopted the ACGIH TWA Value, some have somewhat higher values. Some countries also have a Short Term Exposure Level (STEL) value, usually of 0.21 mg/m^3 (20 ppb).

Doe[45] has recently reviewed the regulatory scene for diisocyanates in the European Economic Community (EEC), Japan and USA, providing information on aspects such as classification, labelling, occupational exposure limits and the position of MDI and TDI in existing chemical legislation. Legislative bodies will be reviewing the available toxicological information on these compounds in future assessments of their hazard and risks. The information assembled in this review indicates the data available for the assessment of the toxicological potential of MDI in many respects which will be of use for hazard and risk evaluations.

REFERENCES

1. Gilbert, D.S., Fate of TDI and MDI in air, soil and water, *J. Cellular Plastics*, 1988; 24: 178-192.

2. International Isocyanate Institute Inc., Unpublished data, stability study of MDI in DMF solution, 1980.

3. Barnard, D., Loadman, M.J.R., and Pepper, B., The stability of phenylisocyanate in dimethylsulphoxide solution, *Tetrahedron Lett.* 1972; 28: 2843-2844.

4. Karol, M.H., and Jim, R., Mechanisms of immunotoxicity to isocyanates, *Chem. Res. Toxicol.* 1991; 4: 503-509.

5. Laboratoire D'Etudes Du Metabolisme Des Medicaments, Unpublished data, pharmacokinetics of MDI after inhalation exposure of rats to labelled MDI, 1977.

6. The Dow Chemical Co., Unpublished data, Metabolic fate of ^{14}C-toluene-2,4-diisocyanate in Fisher 344 rats, 1992.

7. Kennedy, A.L., Stock, M.F., Alarie, Y., and Brown, W.E., Uptake and distribution of ^{14}C during and following inhalation exposure to radioactive toluene diisocyanate, Toxicol. Appl. Pharmacol., 1989; 100: 280-292.

8. Brown, W.E., Kennedy, A.L., Wilson, T.R., Stock, M.F., and Alarie, Y., Interspecies comparison of uptake and distribution of isocyanates following inhalation exposure: Poster presented at the Conference on measuring, understanding and predicting exposures in the 21st century, 1991.

9. International Research & Development Corporation, Unpublished data, acute toxicity studies (LD_{50}) in male albino rats, 1964.

10. The Upjohn Co., Unpuiblished data, acute oral toxicity study on CODE 143L in albino rats, 1982.

11. International Research & Development Corporation, Unpublished data, toluene diisocyanate (TDI) and polymethylene polyphenylisocyanate (PAPI), acute dermal toxicity studies (LD_{50}) in the albino rabbit, 1964.

12. The Upjohn Co., Unpublished data, acute dermal toxicity study on CODE 143L in albino rabbits, 1982.

13. Woolrich, P.F., Toxicology, industrial hygiene and medical control of TDI, MDI and PMPPI, *Am. Ind. Hyg. Assoc. J.*, 1982; 43: 89-97.

X 14. International Research & Development Corporation, Unpublished data, PAPI, MDI, BUNCO, acute inhalation toxicity (LC_{50}) male albino rat, 1965.

X 15. International Isocyanate Institute Inc., Unpublished data, acute inhalation toxicity study of polymeric MDI in rats, 1982.

X 16. International Research & Development Corporation, Unpublished data, toluene diisocyanate (TDI) and polymethylene polyphenylisocyanate (PAPI), six-hour acute inhalation toxicity study in rats, 1964.

17. Weyel, D.A., and Schaffer, R.B., Pulmonary and sensory irritation of diphenyl methane-4,4'- and dicyclohexylmethane-4,4'-diisocyanate, *Toxicol. Appl. Pharmacol.*,1985; 77: 427-433.

18. Duprat, P., Gradiski, D., and Marignac, B., Pouvoir irritant et allergisant de deux isocyanates toluene diisocyanate (TDI), diphenylmethane diisocyanate (MDI), *Europ. J. Toxicol.*, 1976; 9: 41-53.

19. The Upjohn Co., Unpublished data, primary dermal irritation study on CODE 143L in albino rabbits, 1982.

20. The Upjohn Co., Unpublished data, primary eye irritation study on CODE 143L in albino rabbits, 1982.

21. International Research & Development Corporation, Unpublished data, toluene diisocyanate (TDI) and polymethylene polyphenylisocyanate (PAPI), eye irritation test in the albino rabbit, 1964.

22. Tanaka, K., Takeoka, A., Nishimura, F., and Hanada, S., Contact sensitivity induced in mice by methylene bisphenyl diisocyanate, *Contact Dermatitis*, 1987; 17: 199-204.

23. Thorne, P.S., Hillebrand, J.A., Lewis G.R., and Karol, M.H., Contact sensitivity by diisocyanates: potencies and cross-reactivities, *Toxicol. Appl. Pharmacol.*, 1987; 87: 155-165.

24. International Isocyanate Institute Inc., Unpublished data, preliminary study on skin sensitisation caused by MDI solutions, 1980.

25. CIVO Institutes TNO, Unpublished data, preliminary studies of polymeric MDI aerosols and a subacute (2 week) inhalation toxicity study of polymeric MDI in rats, 1983.

26. CIVO Institutes TNO, Unpublished data, a 2 week inhalation study into the mortality of rats as a result of the exposure to polymeric MDI aerosol, 1984.

27. International Research & Development Corporation, Unpublished data, PAPI, TDI, subacute inhalation toxicity study in rats, 1964.

28. Heinrich, U., Koch, W., Schuler, T., Greutzenberg, J., Nolte, T., Hoymann, H.G., Bertsch, W., Preiss, A., and Dasenbrock, C., Inhalation exposure of rats to 4,4'-methylenediphenyl-diisocyanate (MDI); Seventh International Symposium on inhaled particles, 1991.

29. CIVO Institutes TNO, Unpublished data, sub-chronic (13 week) inhalation toxicity study of polymeric MDI aerosol in rats, 1985.

30. CIVO Institutes TNO, Unpublished data, sub-chronic (13 week) inhalation toxicity study of polymeric MDI aerosol in rats, 1986.

31. CIVO Institutes TNO, Unpublished data, chronic toxicity/carcinogenicity inhalation study of polymeric methylenediphenyl diisocyanate aerosol in rats, 1990.

32. Bayer AG, Unpublished data, Desmodur 44M (MDI), salmonella/microsomen-test zur-untersuchung auf punktmutagene wirkung, 1980.

33. Anderson, M., Binderup, M., Kiel, P., Larsen, H., and Maxild, J., Mutagenic action of isocyanates used in the production of polyurethanes, *Scand. J. Work Environ. Health*, 1980; 6: 221-226.

34. Shimizu, H., Suzuki, Y., Takemura, N., Goto, S., and Matsushita, H., The results of microbial mutation test for 45 industrial chemicals, *Japan. J. Ind. Health*, 1985; 27: 400-419.

35. Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., and Speck, W., Salmonella mutagenicity tests: III. results from the testing of 255 chemicals, *Environ. Mutagen.*, 1987; 9: Suppl. 9; 1-110.

36. Inveresk Research International, Unpublished data, testing the mutagenic potential of HE1002 in the mouse lymphoma mutation test, 1981.

37. Inveresk Research International, Unpublished data, testing the mutagenic activity of HE1003 in the mouse lymphoma mutation assay, 1981.

38. Inveresk Research International, Unpublished data, testing of the cell transformation activity of HE1002, 1980.

39. Invesesk Research International, Unpublished data, testing of the cell transformation activity of HE1003, 1980.

40. Japan Chemical Industry Ecology Toxicology & Information Center, Micronucleus test (hexamethylenetetramine, 4,4'-diphenylmethane-diisocyanate), 1982.

41. Karol, M.H., Respiratory effects of inhaled isocyanates, *CRC Crit. Rev. Toxicol.*, 1986; 16: 349-379.

42. Musk, A.W., Peters, J.M. and Wegman, D H., Isocyanates and respiratory disease: current status, *Am. J. Ind. Med.*, 1988; 13: 331-349.

43. Longley, E.O., Methane diisocyanate: A respiratory hazard? *Arch. Environ. Health*, 1964; 8: 898.

44. American Conference of Governmental Industrial Hygienists, *Threshold limit values and biological exposure indices for 1990-1991*, Cincinnati, Ohio, 1990.

45. Doe, J.E., Lyon J.P. and Katoh, M., Regulatory scene for selected diisocyanates, Progress in Rubber & Plastics Technology, 1992; 8: 15-26.

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